

WHAT IS CLAIMED IS:

1. A method for producing a high yield of purified immune globulins from blood plasma, comprising:
  - providing a plasma source containing immune globulins;
  - suspending the immune globulins in an ethanol solution at a volume equivalent to two times that of the initial plasma source at a temperature in a range of about  $-4^{\circ}\text{C}$  to  $-6^{\circ}\text{C}$ ;
  - adjusting the pH of the suspension to about 6.7 to 6.8;
  - incubating the suspension for at least two hours;
  - adding a volume of a solution of about 2.4M glycine in about 7% ethanol and purified water (volume/volume) equivalent to the volume of the plasma source to the suspension;
  - adjusting the pH of the suspension to about 5.2 to 5.4 with 1.0M to 4.0M sodium acetate;
  - extracting the immune globulins using liquid-solid separation;
  - concentrating the protein from the liquid-solid separation by ultrafiltration in a solution of approximately 1.0 gram/deciliter protein content;
  - performing solvent-exchange on the protein solution with a sodium phosphate solution;
  - removing any impurities from the protein solution using an anion exchange chromatography column;
  - concentrating the purified protein deriving from the column effluent by ultrafiltration;
  - inactivating any viruses present in the concentrated protein solution;
  - passing the protein solution through a column containing C-18 resin for removal of remaining residue by adsorption, wherein the ratio of protein load volume to resin volume is

approximately eight parts load volume to one part C-18 resin; and  
formulating the collected protein solution for final use.

2. The method of claim 1, wherein the immune globulins from the plasma source  
5 preferably consist of one of a Cohn fraction II+III and a Cohn fraction I+II+III..

3. The method of claim 1, wherein the ethanol solution is comprised of about 19%  
ethanol and about 81% purified water.

4. The method of claim 1, wherein the suspension of the immune globulins in the  
ethanol solution comprises vigorously agitating the plasma source.

5. The method of claim 1, wherein the suspension of the immune globulins in the  
ethanol solution preferably occurs at a temperature of about -5°C.

6. The method of claim 1, wherein the plasma source containing immune globulins  
is derived from human blood plasma.

7. The method of claim 1, wherein the plasma source containing immune globulins  
comprises the use of non-human sources including those from tissue culture and animal  
origin.

8. The method of claim 1, wherein the volume of the immune globulins suspension  
increases to a volume equivalent to three times that of the initial plasma source to enhance

protein recovery.

9. The method of claim 1, wherein the plasma source contains phospholipids.
10. The method of claim 9, wherein adjusting the pH of the suspension by adding 1M sodium acetate effects precipitation of a majority of the phospholipids as the suspension is continuously agitated.
11. The method of claim 10, wherein the pH of the suspension is adjusted using 4M sodium acetate to yield a lesser volume.
12. The method of claim 1, wherein the step of incubating the suspension for about two hours comprises moderately agitating the suspension.
13. The method of claim 1, wherein the step of adding the solution of glycine and ethanol to the suspension comprises vigorously mixing the suspension.
14. The method of claim 1, wherein the step of adding the solution of glycine and ethanol to the suspension comprises a final concentration of glycine in the suspension of about 8M and a final concentration of ethanol in the suspension of about 15% (volume/volume).
15. The method of claim 1, wherein the step of adjusting the pH of the suspension to about 5.2 to 5.4 with 1.0M to 4.0M sodium acetate comprises increasing the suspension

temperature to approximately  $-2^{\circ}\text{C}$  to  $-3^{\circ}\text{C}$ .

16. The method of claim 1, wherein liquid-separation is performed by one of centrifugation and filtration.
17. The method of claim 1, wherein the step of extracting the immune globulins from the suspension is performed preferably by use of a filter press.
18. The method of claim 1, wherein liquid-separation is facilitated using diatomaceous earth at a concentration of about 1% to about 3% weight by volume during filtration.
19. The method of claim 1, wherein the extraction is performed at a temperature in a range of about  $-2^{\circ}\text{C}$  to  $-3^{\circ}\text{C}$  while moderately agitating the suspension.
20. The method of claim 1, wherein the protein is concentrated by ultrafiltration at a temperature in a range of about  $-2^{\circ}\text{C}$  to  $-3^{\circ}\text{C}$  while moderately agitating the protein solution.
21. The method of claim 1, wherein the protein is filtered through an ultrafilter membrane having a molecular weight cut off of about 100,000.
22. The method of claim 1, wherein solvent-exchange is performed using a solution of about 20mM sodium phosphate at a temperature of about  $5^{\circ}\text{C}$  and a pH of about 6.5.
23. The method of claim 1, wherein the solution for solvent-exchange is prepared by a

mixture of sodium phosphate monobasic and sodium phosphate dibasic at a ratio that yields a pH of about 6.5.

24. The method of claim 1, wherein solvent-exchange is performed using a solution of about 20mM sodium acetate at a pH of about 6.5.

25. The method of claim 1, wherein solvent-exchange is performed by addition of one volume of the pH 6.5 buffer to the protein solution forming a new protein solution and concentrating the new protein solution to its original volume.

26. The method of claim 1, wherein solvent-exchange is performed approximately four times to reduce the alcohol and glycine content.

27. The method of claim 26, further comprising an increase in the temperature of the protein solution to room temperature at 15°C to 25°C after approximately the fourth solvent-exchange.

28. The method of claim 1, wherein the anion exchange chromatography column is equilibrated with a 20mM sodium phosphate buffer at a pH of about 6.5.

29. The method of claim 28, wherein the anion exchange chromatography column is washed with at least one column volume of the 20mM buffer after passing the protein solution therethrough to obtain further protein recovery.

30. The method of claim 1, wherein a ratio of protein to the column is approximately 0.4 grams of protein/milliliter of packed column.
31. The method of claim 1, wherein the purified protein is concentrated from the column effluent to approximately 6 grams/deciliter protein using an ultrafilter membrane having a molecular weight cut off of about 100,000.
32. The method of claim 1, wherein a solvent-detergent method is preferably used for inactivating any viruses present in the concentrated protein solution.
33. The method of claim 32, wherein a mixture of the protein concentrate and solvent-detergent yields a final concentration of 0.3% TNBP (tri-n-butyl phosphate) and 1.0% Triton-X-100 and is incubated for about four hours at about 30°C.
34. The method of claim 1, wherein the step of passing the protein solution through a column containing C-18 resin for removal of remaining residue further comprises adjusting the pH of the protein solution to about 4.6 to about 5.0 with a 4.0M sodium acetate buffer.
35. The method of claim 1, wherein the column containing the C-18 resin is equilibrated with a 20mM sodium acetate buffer at a pH of about 4.6 to about 5.0.
36. The method of claim 1, wherein the collected protein is formulated for final use in one of a liquid and a freeze-dried preparation.

37. The method of claim 36, further comprising the collected protein in the liquid formulation wherein the concentration of the protein is adjusted to a range of about 5.0 to about 10.0 grams/deciliter protein, 0.1% polysorbate-80 (Tween-80), 0.2M glycine, and pH in a range of about 8.2 to about 8.6.

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